MSK.P-038 -2
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rosen et al.
Serial No.: to be assigned
Filing Date: herewith
For : Methods and Compositions for Degradation and/or Inhibition of HER-Family Tyrosine Kinases

PRELIMINARY AMENDMENT

Asst. Commissioner for Patents

Washington, D.C. 20231

Sir:

Preliminary to the calculation of the filing fee for the national phase application which is filed herewith, please make the following amendments:

In the specification:

On Page 1, after the Title, insert:

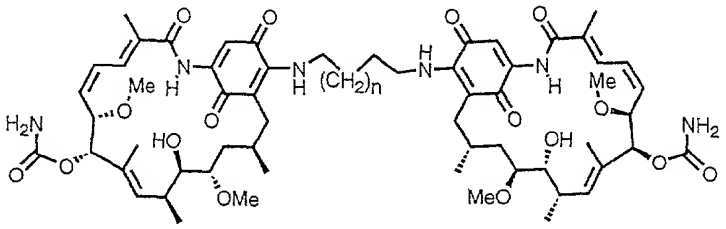
This application is a continuation of International Application No. PCT/US00/09512, filed April 7, 2000, which claims the benefit of US Provisional Application No. 60/128,593 filed April 9, 1999.

On Page 2, please amend the partial paragraph starting on line 21 to read a follows:

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Over-expression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is

associated with a poor prognosis. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

Page 3, please replace the structure at lines 19-24 with the following structure:




**Dimers: n = 1- 9**

Page 5, please replace the first partial paragraph and the first full paragraph with the following:

cause them to undergo homodimerization or heterodimerization with other members of the family. This activates the tyrosine kinase activity of the constituents of the dimer, causes their autophosphorylation and initiates transduction of the mitogenic signal. Although a direct interaction of hsp90 and HER-kinases has not been convincingly demonstrated, the fact that sensitivity of HER2 and other kinases to geldanamycin requires the catalytic domain of the kinase suggests that hsp90 is likely to interact with the catalytic domain of HER-kinases. As HER-kinase heterodimers are quite sensitive to GM, we speculated that each element of the heterodimer interacts with hsp90. Accordingly, it is believed that the dimers of the invention interact with both subunits of the HER-kinase heterodimers and thus more effectively and specifically target the active form of the HER-kinase. The mechanism of action appears to be

based on degradation of the HER-kinases, but may include or in some cases be derived entirely from an inhibition of activity of the HER-kinases.

Fig. 2 shows various compounds which have been synthesized and tested for activity and selectivity as promoters of tyrosine kinase degradation. The compounds tested included geldanamycin, geldanamycin homodimers with linkers of varying lengths, species with quinone or ring-opened geldanamycin linked to geldanamycin and geldanamycin coupled to a linker with no substituent at the other end. The linker in each case is bonded to carbon-17 of the geldanamycin moiety or moieties. The crystal structure of GM bound to hsp90 shows that carbon-17 is the only one not buried in the binding pocket. Stebbins, et al., *Cell* 89, 239-250 (1997).

Page 7, please replace the paragraph starting on line 23 with the following:

GMD-4c was also a potent inhibitor of the growth of breast cancer cells containing HER-kinases (Table 1) with an IC50 of 100 nM against MCF-7 compared to IC50 25 nM for GM and 650 nM for the one-ring opened dimer GMD-a. SKBR3 in which HER2 is highly overexpressed was also found to be very sensitive to GMD-4c. Most epithelial cancer cell lines express one or more members of the HER-kinase family. In order to assess whether the effects of GMD-4c on cells were specific, we utilized the 32D hematopoietic cell line. None of the members of the HER-kinase family are expressed in this murine IL3-dependent myeloid progenitor cell line. Wang, et al., *Proc Natl Acad Sci (USA)* 95, 6809-6814 (1998). GM is a potent inhibitor of 32D; GMD-4c does not appreciably affect its growth.

On Page 8, please replace the first paragraph with the following:

Based on these experimental results, we conclude that GMD-4c induces the selective degradation and/or inhibition of HER-family kinases and specifically inhibits the growth of HER-kinase containing tumor cell lines. This work supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the

presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

On Page 9 of the specification, please replace the partial paragraph starting on line 1 as follows: 100% dimethylsulfoxide (DMSO) and stored at -20°C prior to use in preparation of the geldanamycin derivatives of Fig. 2. The GM analogs were prepared according to the procedure depicted in Fig. 1, which is modified from the method of Schnur et al., *J. Med. Chem.* 38, 3813-3820 (1995). Briefly, the geldanamycin dimers (GMDs) were prepared by treatment of GM with 0.5 eq. of the appropriate diamine in DMSO. The ansa ring-opened GMDs (GMD-a and GMD-aa) were prepared by methanolysis (NaOMe/MeOH) of the GMD-4c. GM-quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

In the Figures:

Please replace figures 1 and 2 with the enclosed replacement sheets.

In the claims:

Please cancel claims 3-5 and 8-11 and amend claims 6, 12 and 13 to read as follows:

6. (amended) The chemical compound of claim [5] 2, wherein the linker has a length of 4 to 7 carbon atoms.

12. (amended) A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with

which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker.

13. (amended) A method for treating cancer in a patient[s] suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker.

Please add claims 15-17 as follows:

15. The method according to claim 13, wherein at least one of the hsp-binding moieties is an ansamycin antibiotic.

16. The method according to claim 15, wherein the linker has a length of 4 to 7 carbon atoms.

17. The method according to claim 16, wherein the linker has a length of 4 carbon atoms.

## REMARKS

This application is a continuation of PCT/US00/09512. In the International Preliminary Examination Report issued by the IPEA/US the Examiner indicated that claims 3-7 and 9-14 met the requirements of PCT Article 33(2)-(4). These claims are being pursued in the a concurrently filed. The present application is directed to the subject matter which was not considered in the PCT application.

No search report was drawn with respect to claims 1, 2 and 8, and thus no International Preliminary Examination Report was issued for these claims, because the Examiner asserted that there was no structural makeup for the claims. Applicants under stand that this is an objection based upon the use of a functional definition for the invention. The examiner's attention is directed to MPEP § 2173.05 where it is stated that "there is nothing inherently wrong with defining some part of an invention in functional terms."

In addition, Applicants have made some minor amendments to the specification. These amendments add a reference to the parent PCT application, and correct several grammatical and clerical errors. No new matter is added by these amendments.

With respect to the replacement figure on Page 3, the replacement adds a double bond which was inadvertently omitted from one of the geldanamycin moieties, but which is included in the other. Since the specification clearly identifies the two parts of this molecule as being the same, and since the structure of geldanamycin was known, this is merely a clerical correction and is not new matter.

Applicants also enclose proposed replacement sheets for Figures 1 and 2, on which the proposed changes are highlighted. In Fig. 1, the changes include the addition of the missing double bond as noted above, changing the view so that the amide groups are more clearly depicted, and adding a subscript to the reagent $H_2N(CH_2)NH_2$ so that it correctly reads $H_2N(CH_2)_7NH_2$. The person skilled in the art would understand that a subscript had been omitted, and that the correct value is 7 since the product formed in the figure has a seven carbon linker. In Fig. 2, the changes alter the view so that the amide groups are more clearly depicted,

and expand the carboxyl groups. It also indicates omitted orientation on one hydroxyl group, consistent with the orientation in the parent molecule. No new matter is added by these corrections.

Respectfully submitted,

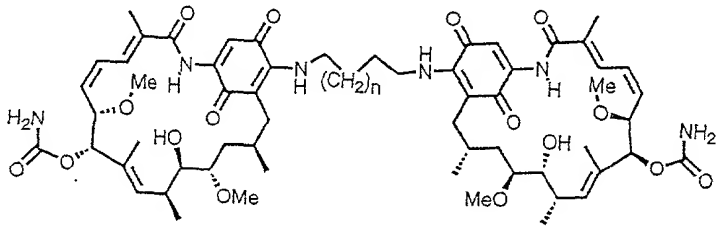
Marina T. Larson
Patent Office Reg. No. 32,038
Attorney for Applicants
(970) 468-6600 x 152

Marked Up Copy Showing Amendments

*On Page 2 of the specification:*

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Over-expression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is associated with a poor prognosis[2]. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

*On Page 3 of the specification*




Dimers: n = 1- 9

*On Page 5 of the specification*

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Based on these experimental results, we conclude that GMD-4c induces the selective degradation and/or inhibition of HER-family kinases and [the] specifically inhibits the growth of HER-kinase containing tumor cell lines. This work[s] supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

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quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

In the claims:

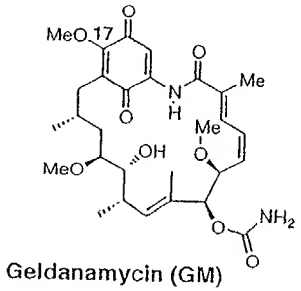
3. (amended) A [The] chemical compound[according to claim 2] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

6. (amended) The chemical compound of claim [5] 4, wherein the linker has a length of 4 to 7 carbon atoms.

9. (amended) The chemical compound of claim [8] 3, wherein the first and second hsp-binding moieties are geldanamycin.

12. (amended) A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker.

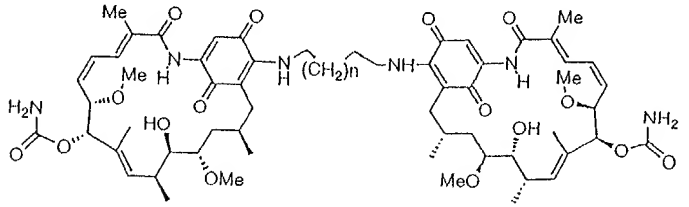
13. (amended) A method for treating cancer in a patient[s] suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.



Geldanamycin (GM)

Diamine (0.49 eq.)

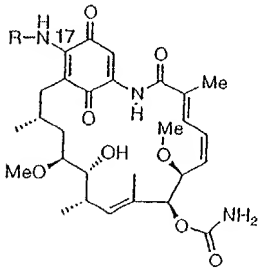
DMSO, dark, 2-24 hrs
> 80% yield

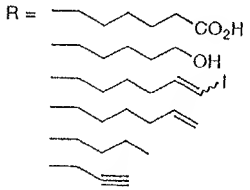


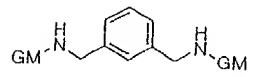
Dimers: n = 1-9

FIG. 1

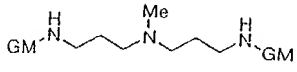
R-$NH_2$ (Excess)
$CHCl_3$, dark



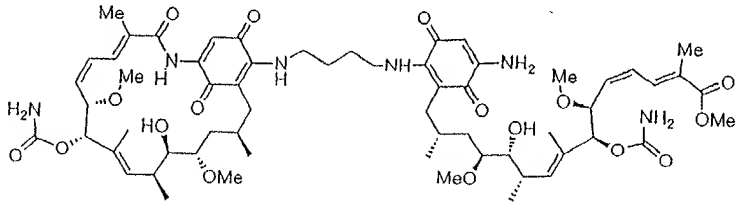




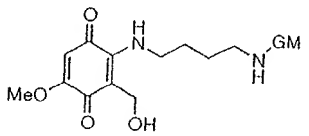
Aryl linkers



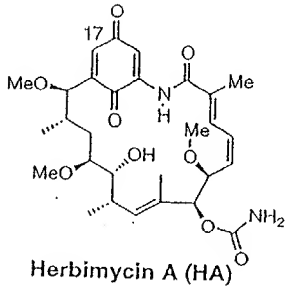
N-methylamino-linker



Geldanamycin Four Carbon
Dimer-Semi-Ansa-Ring Cleaved



GM-Quinone

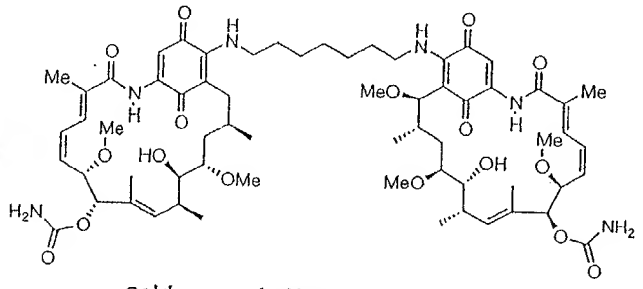


Herbimycin A (HA)

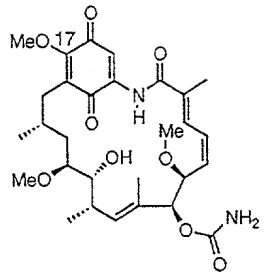
GM

1) $H_2N(CH_2)_7NH_2$, $CHCl_3$

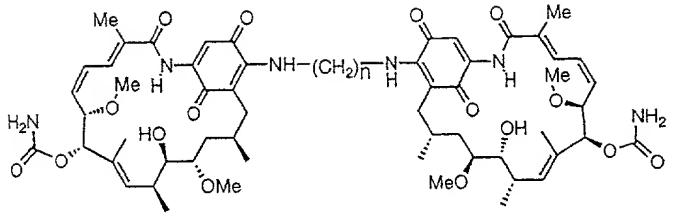
2) HA, $CHCl_3$, Heat, 2 hrs



Geldanamycin-Herbimycin A Heterodimer



Geldanamycin (GM,O)



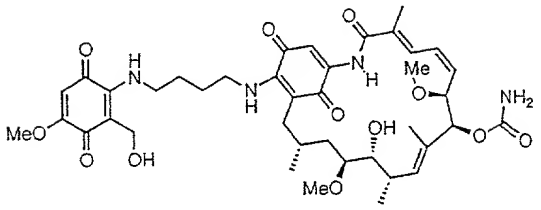
GM dimers (GMD, O—O)

n = 4: GMD-4C, O—(4)—O

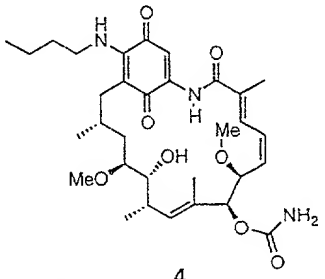
n = 7: GMD-7C, O—(7)—O

n = 9: GMD-9C, O—(9)—O

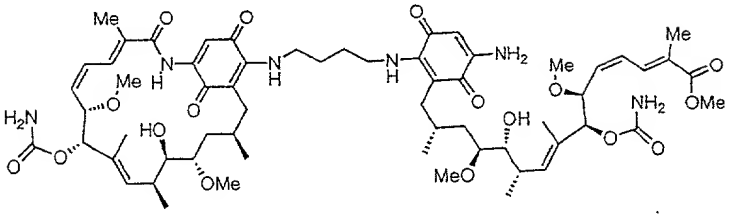
n = 12: GMD-12C, O—(12)—O



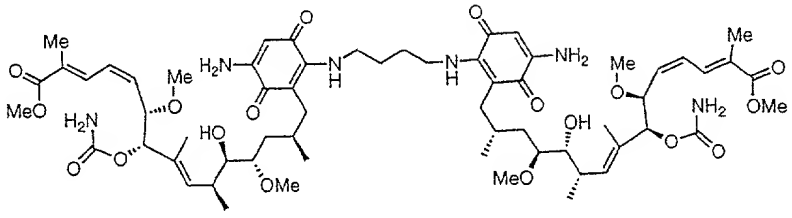
GM-Quinone, O—(4)—□



GM-linker, O—(4)—



GMD-a, O—(4)—Y



GMD-aa, Y—(4)—Y

Fig. 2